

BCM 101

BIOCHEMISTRY

Week 4 Practical

“Chemistry of proteins”

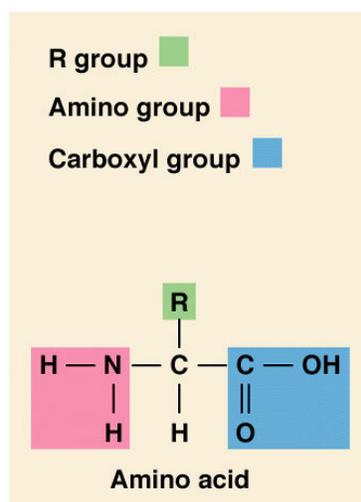
The word “**protein**” is derived from the Greek word “*proteios*”, which means “of primary importance”. In fact, proteins play an important role in all biochemical and physiological body processes; they act as enzymes, hormones, receptors, antibodies and are required for the structural integrity of cells.

The aim of this practical session is to:

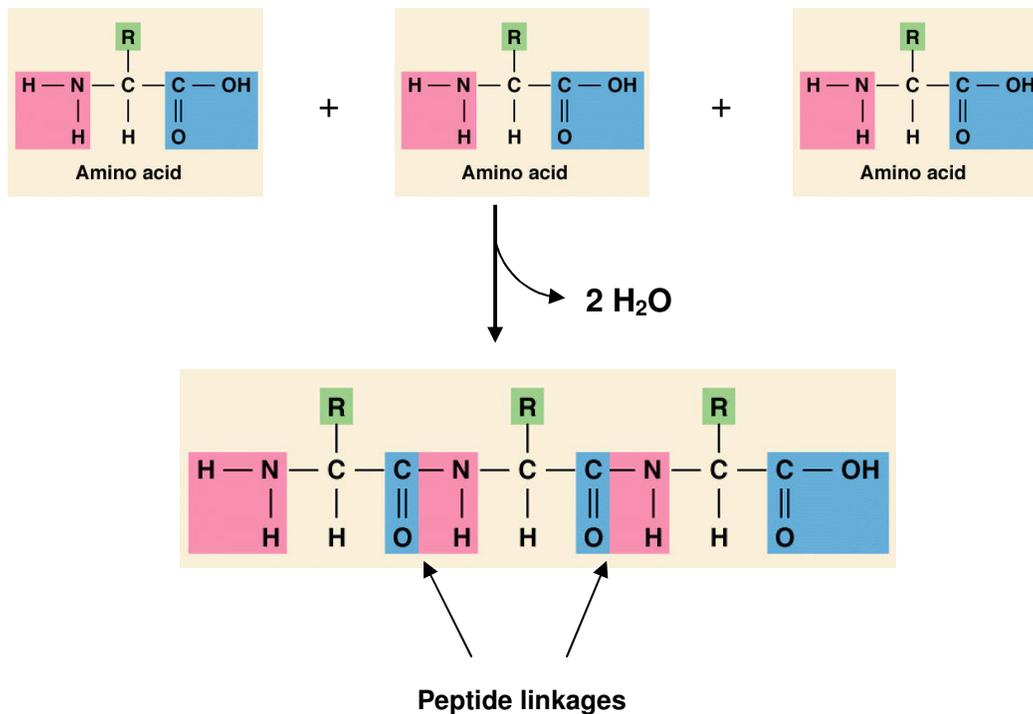
1. Obtain a simplified knowledge about protein structure.
2. Practically apply this knowledge by performing some protein color and precipitation reactions.

Protein structure

Proteins are organic compounds made of “**amino acids**” joined together by “**peptide linkages**”.



These **peptide linkages** are obtained by condensation reactions (removal of water) between carboxylic & amino groups of two adjacent amino acids.



Essential and non-essential amino acids:

There are 20 standard amino acids which differ in their side chain (**R**). Some of them are considered “**essential**” since they cannot be synthesized in our body and must be therefore provided in the diet (e.g. tryptophan & phenylalanine), while others are “**non-essential**” and can be synthesized in the body (e.g. alanine & cysteine).

Functions of dietary proteins:

Proteins are necessary components in our diet. Through the process of digestion, proteins are hydrolyzed into amino acids that can be used for the synthesis of different body proteins (enzymes, hormones, antibodies,...etc), tissue repair and growth. Deficiency of proteins can cause general weakness, protein malnutrition diseases, and decreased resistance to infection.

ANIMAL SOURCES



PLANT SOURCES



Practical

Using the provided solutions of **albumin** (egg white), **casein** (milk protein) and **gelatin** (animal collagenous material), perform the following:

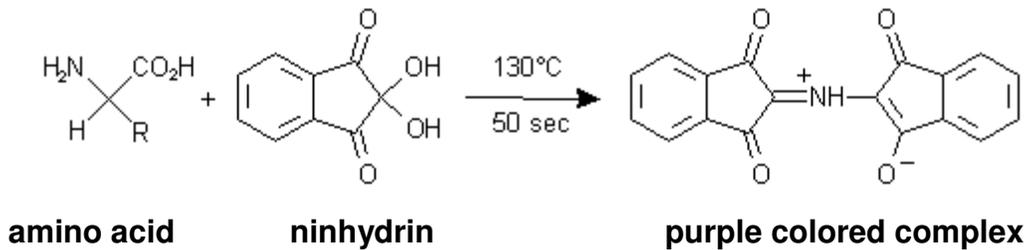
- A. General tests**
- B. Color reactions**
- C. Precipitation reactions**

A. General tests for proteins

1. Ninhydrin reaction:

Principle:

Ninhydrin reacts with amino acids in proteins at high temperature giving a purple colored complex.



Ninhydrin is most commonly used as a forensic chemical to detect “**fingerprints**”, as amines left over from proteins sloughed off in fingerprints react with ninhydrin giving a characteristic purple color.



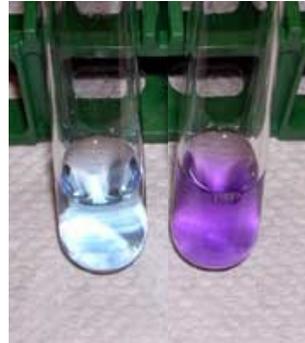
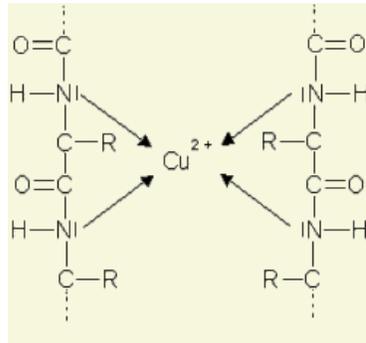
Procedure & observation:

- To 1 ml amino acid solution in a test tube, add 1 drop of ninhydrin.
- Put in a boiling water bath and observe the formation of a purple color.

2. Biuret test:

Principle:

The biuret reagent (copper sulfate in a strong base) reacts with peptide bonds in proteins to form a blue to violet complex known as the “**biuret complex**”. **N.B.** Two peptide bonds at least are required for the formation of this complex.



Biuret complex

Procedure & observation:

- To 2 ml of protein solution in a test tube, add 3 drops of 10% sodium hydroxide solution and 3-6 drops of 0.5% copper sulfate solution.
- Mix well; a blue to violet color is obtained with albumin, casein & gelatin.

B. Color reactions of proteins

1. Reduced sulfur test:

Principle:

Proteins containing sulfur (in **cysteine** and **cystine**) give a black deposit of lead sulfide (PbS) when heated with lead acetate in alkaline medium.

Procedure & observation:

- To 1 ml of protein solution in a test tube, add 2 drops of 10% sodium hydroxide solution and 2 drops of lead acetate.
- Mix well and put in a boiling water bath for few minutes; a black deposit is formed with albumin, while a slight black turbidity is obtained with casein due to its lower content of sulfur. Gelatin gives negative result.

2. Xanthoproteic acid test:

Principle:

Nitric acid gives a color when heated with proteins containing **tyrosine** (yellow color) or **tryptophan** (orange color); the color is due to nitration.

Procedure & observation:

- To 2 ml of protein solution in a test tube, add 2 drops of concentrated nitric acid.
- A white precipitate is formed and upon heating in a boiling water bath, it turns yellow with “**tyrosine**” and orange with the essential amino acid “**tryptophan**” indicating a high nutritive value.

3. Millon's test:

Principle:

Millon's reagent (Hg/HNO_3) gives positive results with proteins containing the phenolic amino acid “**tyrosine**”.

Procedure & observation:

- To 2 ml of protein solution in a test tube, add 3 drops of Millon's reagent.
- Mix well and heat directly on a small flame.
- A white ppt is formed with albumin and casein (but not gelatin); the ppt gradually turns into brick red.

4. Hopkins-Colé test:

Principle:

Hopkins-Colé reagent (magnesium salt of oxalic acid) gives positive results with proteins containing the essential amino acid “**tryptophan**” indicating a high nutritive value.

Procedure & observation:

- To 1 ml of protein solution in a test tube, add 1 ml of Hopkins-Colé reagent and mix well.
- Incline the test tube and slowly add 1 ml of concentrated H_2SO_4 on the inner wall of the test tube to form 2 layers.
- Put the test tube in a boiling water bath for 2 minutes.
- A reddish violet ring is formed at the junction between the 2 layers with albumin and casein; gelatin gives negative results.

C. Precipitation reactions of proteins

1. Precipitation by heavy metals:

Principle:

Heavy metals (e.g. Hg^{2+} , Pb^{2+} , Cu^{2+}) are high molecular weight cations. The positive charge of these cations counteracts the negative charge of the carboxylate group in proteins giving a precipitate.

Procedure & observation:

- To 1 ml of protein solution in a test tube, add 1 drop of lead acetate; a white ppt is obtained.
- To 1 ml of protein solution in a test tube, add 1 drop of 10% copper sulfate; a blue ppt is obtained.

2. Precipitation by alkaloidal reagents:

Principle:

Alkaloidal reagents (e.g. **tannate** & **trichloroacetate**) are high molecular weight anions. The negative charge of these anions counteracts the positive charge of the amino group in proteins giving a precipitate.

Procedure & observation:

- To 1 ml of protein solution in a test tube, add tannic acid drop wise until a buff ppt is obtained.
- To 1 ml of protein solution in a test tube, add 1 ml of trichloroacetic acid (TCA); a white ppt is obtained.

N.B. Precipitation of proteins by heavy metals and alkaloidal reagents indicates the presence of both negative and positive charges and hence the **amphoteric** nature of proteins.

3. Precipitation by denaturation:

a. Denaturation by heat (heat coagulation test):

Principle:

Heat disrupts hydrogen bonds of secondary and tertiary protein structure while the primary structure remains unaffected. The protein increases in size due to denaturation and coagulation occurs.

Procedure & observation:

- Put 2 ml of protein solution in a test tube, incline it and heat to boiling.
- A permanent clotting and coagulation is obtained with **albumin** only.

b. Denaturation by acids (Heller's test):

Principle:

Nitric acid causes denaturation of proteins with the formation of a white ppt (this differs from the nitration reaction in "xanthoproteic acid test").

Procedure & observation:

- Put 2 ml of concentrated nitric acid in a test tube.
- Incline the tube and slowly add 1 ml protein solution drop wise to form a layer above the nitric acid layer.
- A white ring is formed at the interface between the 2 layers.

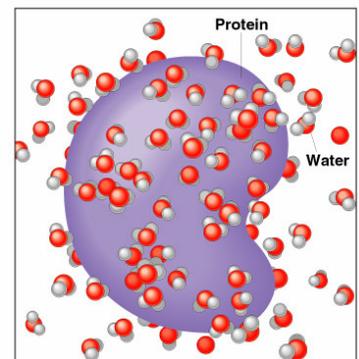
4. Fractional precipitation by ammonium sulfate (salting out):

Principle:

Protein molecules contain both hydrophilic and hydrophobic amino acids. In aqueous medium, hydrophobic amino acids form protected areas while hydrophilic amino acids form hydrogen bonds with surrounding water molecules (**solvation layer**).

When proteins are present in **salt** solutions (e.g. ammonium sulfate), some of the water molecules in the solvation layer are attracted by salt ions. When salt concentration gradually increases, the number of water molecules in the solvation layer gradually decreases until protein molecules coagulate forming a precipitate; this is known as "**salting out**".

As different proteins have different compositions of amino acids, different proteins precipitate at different concentrations of salt solution.



Procedure & observation:

- To 2 ml of egg-white solution (containing both albumin & globulin), add an equal volume of saturated ammonium sulfate solution; globulin is precipitated in the resulting half saturated solution of ammonium sulfate.
- Separate globulin by centrifugation and recover the clear supernatant.
- Add ammonium sulfate crystals gradually to the clear supernatant until full saturation occurs; another precipitate (albumin) is obtained.
- Separate albumin by centrifugation.

N.B. The reason for the precipitation of globulin and albumin at different ammonium sulfate concentration could be that the solvation layer around globulin is looser and thinner than that around albumin. Therefore, globulin needs only half-saturated ammonium sulfate to lose its solvation layer while albumin loses its solvation layer in a fully saturated ammonium sulfate solution.

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Student Name: **Student number:**

Laboratory exercise:

- Using the provided solutions of **albumin**, **casein** and **gelatin** perform the tests in the table below and write down your observations.

	Albumin	Casein	Gelatin
Biuret test			
Reduced sulfur test			
Xanthoproteic acid test			
Heavy metal pptn			
Alkaloidal reagent pptn			
Heat coagulation test			
Heller’s test			

- Is gelatin of high nutritive value? Why?
.....
- Observe the demonstration of fractional separation of albumin and globulin from egg white using ammonium sulfate (salting out).